Since it was difficult to obtain directly in a single experiment films of exactly 0.1-mm. thickness, several films were selected so that the thickness of each film might be distributed in a range from 0.05 to 0.4 mm. and the respective water vapor permeability was measured at various humidity conditions. Plotting the relationship between permeation resistance ($1/Q$) and film thickness (l) gave a straight line. Thus, the permeability (Q) of the film of a given thickness (l) in various conditions could be obtained.

Figure 1 suggests some noteworthy points. According to Eq. 2, the relationship between Q ($=q/A$) and Δp ($=p_1 - p_2$) should be linear and proportional. As shown in Fig. 1, however, most of the obtained curves do not agree with Eq. 2. In particular, the value of Q for PVA does not increase after the maximum point, even when the value of Δp increases. The cause of this apparently controversial phenomenon may be that the permeability, Q, depends not only on the pressure difference between the higher vapor pressure side and the lower side, but also on the mean humidity condition to which the test film is subjected. This feature may be called the water vapor pressure dependency of permeability.

The permeability coefficient (P) for a hydrophobic film is constant, because water vapor does not interact with the film, and the experimental data correspond to Eq. 1 or 2. On the other hand, for hydrophilic films such as coating films, the value of P changes according to the humidity condition, because water vapor interacts with the film; thus Eq. 1 or 2 does not apply to the hydrophilic film. The relationship between P and Δp was obtained from Eq. 2 by using the data of Fig. 1. Figure 2 shows the results obtained. The value of P changed sharply, especially for the PVA film.

Figure 3 illustrates the relationship between Q and Δp for the double-layer film, which has the remarkable two-sided feature of permeation. For comparison, this figure also shows the curves obtained with each single-component film. The permeability Q of the single films and of the double-layer film is expressed on the basis of 0.1-mm. thickness. It was found (Fig. 3) that two-sided features, classified as Group α and Group δ (1), occurred over different humidity ranges. These features will, of course, change according to the sort of double-layer film or the combination of single films used therein.

SUMMARY

With both single-component films and a double-layer film, the relationship between water vapor permeability (Q) and humidity condition was studied. It was found that the permeability of the films depended not only upon the difference in vapor pressure (Δp) between the higher and lower humidity sides, but also upon the mean humidity condition to which the test film was subjected. It was also found that a double-layer film with two-sided permeability displayed the feature of Group α in a certain humidity range and the feature of Group δ in another humidity range.

REFERENCES

(1) T. Kuriyama, M. Nobutoki, and M. Nakanishi, *J. Pharm Sci.*, **59**, 1341 (1970).

(2) K. W. Ninneman and L. E. Simerl, *Pulp Pap. Mag. Can.*, **63**, T68(1962).

(3) B. Takeda, *Nippon Kagaku Zasshi*, **74**, 207(1953).

(4) C. E. Rogers, V. Stannet, and N. Szwarc, *Ind. Eng. Chem.*, **49**, 1933(1957).

(5) M. Patel, J. M. Patel, and A. P. Lemberger, *J. Pharm. Sci.*, **53**, 286(1964).

ACKNOWLEDGMENTS AND ADDRESSES

Received November 12, 1969, from the *Research Laboratories of Yoshitomi Pharmaceutical Industries, Ltd., Yoshitomi-Cho, Chikujo-Gun, Fukuoka-Prefecture, Japan.*

Accepted for publication March 10, 1970.

Abstracted in part from a thesis submitted by T. Kuriyama to the Graduate School, Kyushu University, in partial fulfillment of Doctor of Philosophy degree requirements.

Thanks are expressed to Professor S. Iguchi for his interest in the work and useful discussions. The authors also thank Mr. M. Tasaka, Executive Director, Yoshitomi Pharmaceutical Industries, Ltd., for his encouragement and his kind permission to publish this paper.

GLC Determination of Guaiacol Glyceryl Ether in Blood

WILLIAM R. MAYNARD, Jr., and ROBERT B. BRUCE

Abstract □ A method has been developed for the determination of guaiacol glyceryl ether in blood by extraction with methylene chloride followed by conversion to the heptafluorobutyrate ester and quantitation using an electron-capture detector. Blood levels following oral administration of the drug indicate a rapid absorption and elimination with a half-life of 1 hr.

Keyphrases □ Guaiacol glyceryl ether—GLC determination, in blood □ NMR—identification □ GLC—determination, guaiacol glyceryl ether, in blood

Guaiacol glyceryl ether (GGE) has been used for many years as an expectorant and, more recently, it has been claimed to have activity as a muscle relaxant (1) and as an hypocholesteremic (2–5), and to reduce platelet adhesiveness (6). In spite of its extensive use and study, no methods appear to be available for its determination in man following usual dosages. Morgan *et al.* (7) studied blood levels in dogs, and Mizutani and Naito (8) have determined blood levels in rabbits. However, relatively large doses were administered in each case, and these methods do not appear adaptable to present needs. Therefore, a method has been developed using GLC which appears to be satisfactory.

EXPERIMENTAL

The method is based on extraction of GGE from blood with methylene chloride and conversion to the heptafluorobutyrate ester and quantitation by GLC using an electron-capture detector. Mephenesin [3-(*o*-toloxy)-1,2-propanediol] is used as an internal standard.

The procedure is carried out as follows. To 5.0 ml. of blood, add 1.0 mcg. of mephenesin, 3 ml. of distilled water, and 0.5 ml. of 2 *N* H_2SO_4. Then extract with 20 ml. of redistilled methylene chloride by shaking for 10 min. Separate the phases by centrifuging, and dry the methylene chloride extract by passing it through a layer of anhydrous sodium sulfate in a funnel. Repeat the extraction with an

Table I—Recovery of Known Amounts of GGE Added to Control Blood

GGE—mcg./ml. Blood— Added	Found	Recovery, %
0.080	0.078	97.5
0.16	0.15	92.5
0.20	0.19	97.0
1.00	1.02	102.0
2.00	1.89	94.4
3.00	3.07	101.0

additional 10 ml. of methylene chloride, and combine the extracts. Wash sodium sulfate with an additional 3 ml. of methylene chloride which is added to the combined extract. Evaporate the combined extracts under a stream of nitrogen to 100 μl. Add 300 μl. of a solution of heptafluorobutyric anhydride in methylene chloride (1 ml./100 ml.), mix, heat to 60° for 30 sec., and allow to stand for 10 min. with occasional mixing. Evaporate to complete dryness with nitrogen, add 0.50–3.0 ml. of redistilled ether, mix, and inject 1–4 μl. into the gas chromatograph.

The gas chromatograph used in this study was a Barber-Coleman, Series 5000, with a ^{63}Ni-detector. The column was 1.21 m. (4 ft.) long, stainless steel, and contained 3% XE-60 on diatomaceous earth.[1] Temperatures were: column, 144°; detector, 280°; and injection port, 260°. The flow rate was 40 ml./min. of nitrogen. The retention time of mephenesin heptafluorobutyrate was 4 min. 50 sec. and that of GGE heptafluorobutyrate was 6 min. 10 sec. The column was preconditioned by four rapid injections of 4 μl. of a mixture of heptafluorobutyric anhydride in ether (1:4) and

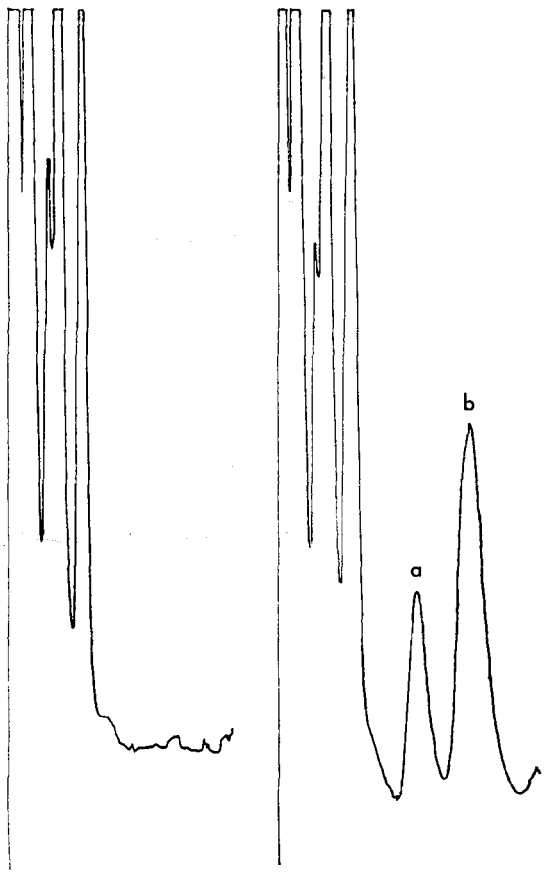


Figure 1—*Chromatograms showing results of the analysis of control blood (left) and blood sample from subject receiving GGE (right): a, mephenesin heptafluorobutyrate; and b, GGE heptafluorobutyrate.*

[1] Gas-Chrom Q, Applied Science Laboratories, Inc., State College, PA 16801

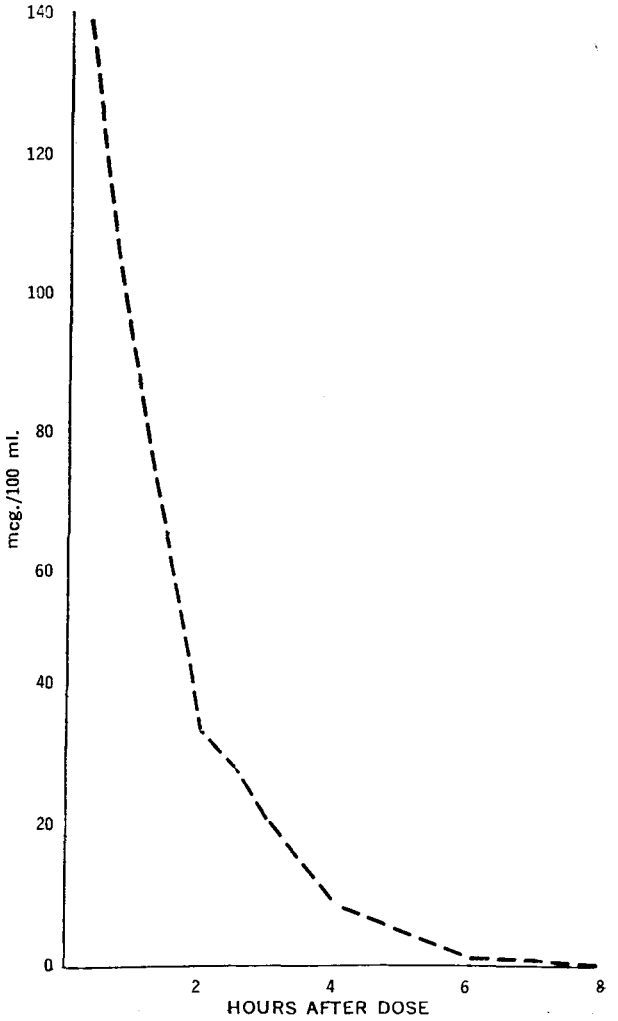


Figure 2—*Average blood levels following the oral administration of 600 mg. of GGE to three human subjects.*

allowing the column to remain at 144° for 30 min. The column temperature was raised to 200° for 2 hr. The column exit was disconnected during this preconditioning.

Three normal male subjects were administered an oral liquid dose of 600 mg. of GGE. The subjects received no solid food from the midnight before drug administration until 2 hr. after drug administration. Blood samples were drawn at frequent intervals for 8 hr. after the dose.

RESULTS AND DISCUSSION

GGE has been determined (9, 10) by GLC in pharmaceutical preparations. These methods did not give the sensitivity needed for blood level determination. Amides and esters prepared from halogenated acid chlorides or anhydrides give a high response with the electron-capture detector. Heptafluorobutyric anhydride was used because it reacts easily and quickly with GGE at room temperature and can be readily separated from the ester by simple evaporation. The procedure is straightforward and simple to carry out. The only precaution is to remove completely the solvent—methylene chloride—before the sample is injected into the gas chromatograph, since the detector is very sensitivie to halogenated compounds.

The results found from the analysis of blood samples to which known amounts of GGE had been added are shown in Table I. The standard deviation at the 1.00-mcg./ml. level was 0.046. These results appear to be satisfactory for the analysis of blood. Chromatograms from actual analysis of blood samples are shown in Fig. 1. A comparison of the chromatogram of the control blood with that of the sample containing GGE shows that nothing present in

the control interferes with the determination. Some uninvestigated materials are eluted earlier than the esters of GGE and mephenesin. Quantitation is made comparing peak heights of the internal standard with that of the GGE ester.

A large amount of the ester was prepared by the procedure described to determine whether reaction had occurred with both or only one of the hydroxyl groups. NMR spectra showed that only the primary hydroxyl had reacted.

The results from the blood level determinations following the oral dose are shown in Fig. 2. GGE is readily absorbed, with the maximum amount determined occurring in the 0.25-hr. sample. The half-life was 1.00 hr. Detectable amounts of the drug were no longer present in the 8-hr. samples of any of the subjects, indicating rapid metabolism and excretion.

REFERENCES

(1) H. Hafner, *Wien. Med. Wochenschr.*, **114**, 523(1964).

(2) R. D. Eastham and E. P. Griffiths, *Lancet*, **7441**, 795(1966).

(3) J. L. Silverman and H. A. Wurzel, *Amer. J. Med. Sci.*, **254**, 491(1967).

(4) H. R. Baumgartner, M. Cronquist, P. Wobman, and F. Streuli, *Schweiz. Med. Swchr.*, **97**, 1674(1967).

(5) J. Franco and L. R. Grams, *Clin. Res.*, **16**, 41(1968).

(6) S. Naito and M. Mizutani, *Chem. Pharm. Bull.*, **17**, 822 (1969).

(7) A. M. Morgan, E. B. Truitt, Jr., and J. M. Little, *J. Amer. Pharm. Ass., Sci. Ed.*, **46**, 374(1957).

(8) M. Mizutani and S. Naito, *Chem. Pharm. Bull.*, **15**, 1422 (1967).

(9) B. R. Rades and E. S. Aranda, *J. Pharm. Sci.*, **57**, 847(1968).

(10) M. Koibuchi, T. Shibazaki, T. Minamakawa, and Y. Nishimura, *J. Pharm. Soc. Jap.*, **88**, 877(1968).

ACKNOWLEDGMENTS AND ADDRESSES

Received February 10, 1970, from the *Research Laboratories, A. H. Robins Company, Inc., Richmond, VA 23220*

Accepted for publication March 18, 1970.

The authors thank Dr. Russell C. Seckendorf, Clinical Pharmacology, A. H. Robins Company, for the administration of the drug and collection of blood samples.

Antiradiation Compounds XIV: Dithiocarbamates of Aminothiophenes

WILLIAM O. FOYE, JAMES MICKLES, and GERARD M. BOYCE

Abstract □ Dithiocarbamates of 2-amino-3-cyano(or carbethoxy)-4,5-dialkylthiophenes and a corresponding furan have been obtained. Dithiocarbamate formation of 2-amino-3-cyano-4,5-diphenylfuran resulted in a conversion to the corresponding thiophene dithiocarbamate. A dithiocarbamate trithiocarbonate of 2-amino-3-carbethoxy-4-mercaptomethylthiophene was also synthesized, and ring closure of the 2-amino-3-cyanothiophenes to thiopheno[2,3-*d*]pyrimidines was observed. None of the compounds tested showed radiation-protective or antimalarial properties.

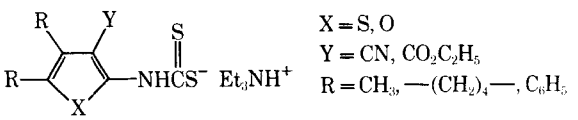
Keyphrases □ Antiradiation compounds—dithiocarbamates of aminothiophenes □ Aminothiophenes, dithiocarbamate derivatives—radiation-protective capacity, antimalarial properties □ IR spectrophotometry—structure

Although thiophene derivatives have not appeared frequently with radiation-protective properties, a basic derivative, *N*-phenyl-2-thiophenecarboxamidine, has been reported to have appreciable protection in rats (1). Since several heterocyclic dithiocarbamates in the pyridine, pyrimidine, and acridine series (2) are radiation protective, dithiocarbamates of thiophenes and furans having basic functions appeared to be logical candidates as radiation-protective compounds. Methods for obtaining thiophenes and furans having primary amino substituents in the ring have recently been announced (3), and the conversion of compounds of this type to dithiocarbamates has been attempted. Inclusion of this sulfur-containing function provides a thiol anion capable of undergoing rapid hydrogen-atom exchange reactions (4), which could account for radiation protection.

PROCEDURE

Preparation of 2-aminothiophenes was carried out by the method of Gewald *et al.* (5) with modifications. This procedure involved the base-catalyzed condensation of a carbonyl compound with an active methylene nitrile and sulfur. Using methyl ethyl ketone and malononitrile, the reaction was found to be best catalyzed with morpholine, with excess ketone as the solvent. Using methyl ethyl ketone and malononitrile, the product was 2-amino-3-cyano-4,5-dimethylthiophene; with ethanol as the solvent, the product was 2-butylidenemalononitrile. By the same procedure, but with ethanol as the solvent, the following were obtained: 2-amino-3-carbethoxy-4,5-dimethylthiophene, 2-amino-3-cyano-4,5-tetramethylenothiophene, and 2-amino-3-carbethoxy-4,5-tetramethylenothiophene. Also obtained by the same general procedure, without sulfur, were 2-amino-3-cyano-4,5-dimethylfuran and the corresponding 4,5-diphenyl compound.

Attempts to form the dithiocarbamates of the 2-aminothiophenes previously mentioned, using carbon disulfide and ethanol as the solvent, gave only small yields over a period of 24–72 hr. In the case of 2-amino-3-cyano-4,5-dimethylthiophene, the thiourea was formed instead. By using the procedure of Fairfull and Peak (6), however, triethylammonium salts of the dithiocarbamates (I) of the aminothiophenes and one of the aminofurans were obtained in good yield and sufficiently pure for analysis.



I

The attempted conversion of 2-amino-3-cyano-4,5-diphenylfuran to the dithiocarbamate gave a product having a poor analysis for the ethyl ester of the dithiocarbamate. By allowing the reaction to take place during a much longer time (2 weeks), a product was obtained for which the analysis indicated formation of the dithiocarbamate salt of 2-amino-3-cyano-4,5-diphenylthio-